Please amend claims 2, 4-6, 8, 9, 12, 14, 15, 18, 20, 22-24 and 27, and add new claims 28-33 to read as follows. A marked-up copy of the amended claims, showing the changes made thereto, is attached.

2. (Amended) A method for quantitatively determining LDL cholesterol in a biological sample, which comprises:

(I) reacting cholesterol in the presence of:

a) a biological sample,

b) CH enzymes selected from the group consisting of (i) a combination of cholesterol esterase and cholesterol oxidase and (ii) a combination of cholesterol esterase and cholesterol dehydrogenase, and

c) a polyoxyethylene derivative and a polyoxyethylene-polyoxypropylene copolymer which enable the CH enzymes to act only on LDL cholesterol to form hydrogen peroxide or reduced coenzyme; and

(II) measuring the amount of the hydrogen peroxide or reduced coenzyme.

4. (Amended) The method according to claim 2, wherein the polyoxyethylene-polyoxypropylene copolymer is a surfactant represented by formula (I):

$$HO—(C_2H_4O)_a—(C_3H_6O)_b—(C_2H_4O)_c—H \quad (I)$$

wherein a, b and c independently represent an integer of 1 to 200.

5. (Amended) A method for continuous fractional determination of HDL cholesterol and LDL cholesterol in a biological sample, which comprises:

(I) subjecting cholesterol to reaction in the presence of:

a) a biological sample,

b) CH enzymes selected from the group consisting of (i) a combination of cholesterol esterase and cholesterol oxidase and (ii) a combination of cholesterol esterase and cholesterol dehydrogenase, and

c) a reagent enabling the CH enzyme to act only on HDL cholesterol to form hydrogen peroxide or reduced coenzyme,

(II) measuring the amount of the hydrogen peroxide or reduced coenzyme to quantitatively determine the concentration of HDL cholesterol, then adding a polyoxyethylene derivative and a polyoxyethylene-polyoxypropylene copolymer which enable the CH enzymes to act only on LDL cholesterol;

(III) subjecting cholesterol to the reaction to form hydrogen peroxide or reduced coenzyme;

(IV) measuring the amount of the hydrogen peroxide or reduced coenzyme to quantitatively determine the concentration of LDL cholesterol.

6. (Amended) A method for continuous fractional determination of HDL cholesterol and LDL cholesterol in a biological sample, which comprises:

(I) conducing a first reaction of cholesterol in the presence of:

a) a biological sample,

b) CH enzymes selected from the group consisting of (i) a combination of cholesterol esterase and cholesterol oxidase and (ii) a combination of cholesterol esterase and cholesterol dehydrogenase, and

c) a reagent enabling the CH enzymes to act only on HDL cholesterol to form hydrogen peroxide or reduced coenzyme, and

(II) measuring the amount of the hydrogen peroxide or reduced coenzyme to quantitatively determine the concentration of HDL cholesterol, then adding

CH enzymes, and a polyoxyethylene derivative and a polyoxyethylene-polyoxypropylene copolymer which enable the CH enzymes to act only on LDL cholesterol,

(III) conducting a second reaction of cholesterol to form hydrogen peroxide or reduced coenzyme, and measuring the amount of the hydrogen peroxide or reduced coenzyme to quantitatively determine the concentration of LDL cholesterol.

8. (Amended) The method according to claim 5 or 6, wherein the polyoxyethylene derivative is a polyoxyethylene alkyl ether or a polyoxyethylene alkylaryl ether.

9. (Amended) The method according to claim 5 or 6, wherein the polyoxyethylene-polyoxypropylene copolymer is a surfactant represented by formula (I):

$$HO—(C_2H_4O)_a—(C_3H_6O)_b—(C_2H_4O)_c—H \quad (I)$$

wherein a, b and c, independently represent an integer of 1 to 200.

12. (Amended) The method according to claim (5) or (6), wherein the reagent enabling CH enzyme to act only on HDL cholesterol is a reagent for aggregating lipoproteins other than HDL.

14. (Twice Amended) The method according to claim (12), wherein the reagent for aggregating lipoproteins other than HDL is a reagent comprising a divalent metal salt and at least one member selected from the group consisting of heparin or a salt thereof, phosphotungstic acid or a salt thereof, dextran sulfuric acid or a salt thereof, polyethylene glycol, sulfated cyclodextrin or a salt thereof, and sulfated oligosaccharide or a salt thereof.

15. (Twice Amended) The method according to claim (6), wherein the CH enzymes used in the first reaction are chemically modified enzymes and the CH enzymes used in the second reaction are enzymes that are not chemically modified.

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18. (Amended) A reagent for determining LDL cholesterol comprising CH enzymes selected from the group consisting of (i) a combination of cholesterol esterase and cholesterol oxidase and (ii) a combination of cholesterol esterase and cholesterol dehydrogenase, and a polyoxyethylene derivative and a polyoxyethylene-polyoxypropylene copolymer which enable the CH enzymes to act only on LDL cholesterol.

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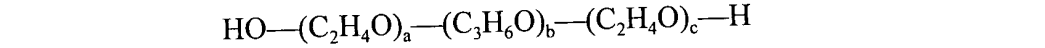
20. (Amended) The reagent according to claim (18), wherein the polyoxyethylene-polyoxypropylene copolymer is a surfactant represented by formula (I):



$$HO—(C_2H_4O)_a—(C_3H_6O)_b—(C_2H_4O)_c—H \qquad (I)$$

wherein a, b and c independently represent an integer of 1 to 200.

B8

22. (Amended) A reagent kit for continuous fractional determination of HDL cholesterol and LDL cholesterol comprising a first reagent comprising CH enzymes selected from the group consisting of (i) a combination of cholesterol esterase and cholesterol oxidase and (ii) a combination of cholesterol esterase and cholesterol dehydrogenase, and a reagent for aggregating lipoproteins other than HDL, and a second reagent comprising a polyoxyethylene derivative and a polyoxyethylene-polyoxypropylene copolymer which enable CH enzymes to act only on LDL cholesterol.

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23. (Amended) The reagent kit according to claim 22, wherein the polyoxyethylene derivative is a polyoxyethylene alkylaryl ether.

24. The reagent kit according to claim (22), wherein the polyoxyethylene-polyoxypropylene copolymer is a surfactant represented by formula (I):

$$HO—(C_2H_4O)_a—(C_3H_6O)_b—(C_2H_4O)_c—H \quad (I)$$

wherein a, b and c independently represent an integer of 1 to 200.

27. (Three Times Amended) The reagent kit according to claim 22, wherein the reagent for aggregating lipoprotein other than HDL is a reagent comprising a divalent metal salt and at least one member selected from the group consisting of heparin or a salt thereof, phosphotungstic acid or a salt thereof, dextran sulfuric acid or a salt thereof, polyethylene glycol, sulfated cyclodextrin or a salt thereof, and sulfated oligosaccharide or a salt thereof.

28. (New) The method according to claim 2, 5 or 6, wherein the CH enzymes are cholesterol esterase and cholesterol oxidase, and the determination of hydrogen peroxide is carried out by reacting the hydrogen peroxide with chromogen in the presence of peroxidase to form a dye and measuring the absorbance of the reaction mixture.

29. (New) The reagent according to claim 18, wherein the CH enzymes are cholesterol esterase and cholesterol oxidase, and the reagent further comprises peroxidase and chromogen capable of producing a dye by reaction with hydrogen peroxide in the presence of peroxidase.

30. (New) The reagent kit according to claim 22, wherein the second reagent further comprises CH enzymes.

31. (New) The reagent kit according to claim 22, wherein the CH enzymes are cholesterol esterase and cholesterol oxidase, and the first reagent further comprises peroxidase and chromogen capable of producing a dye by reaction with hydrogen peroxide in the presence of peroxidase.

32. (New) The reagent kit according to claim 30, wherein the CH enzymes in the first reagent are chemically modified enzymes and the CH enzymes in the second reagent are enzymes that are not chemically modified.

R 11 Cont.

33. (New) The method according to claim 12, wherein the reagent for aggregating lipoproteins other than HDL further contains a nonionic surfactant that does not solubilize the aggregated lipoproteins.

REMARKS

Claims 2, 5 and 6 have been amended in order to recite the present invention with the specificity required by statute. Claims 18 and 22 have been rewritten in independent form and claims 2, 4, 8, 9, 12, 14, 15, 20, 23, 24 and 27 have been amended to better depend from their antecedent claims. Additionally, new Claims 28-33 are presented in order to more specifically recite various preferred embodiments of the present invention and claims 1, 7, 10, 11, 16, 17, 21, 25 and 26 have been canceled in order to reduce the issues. No new matter has been added.

Claims 1-9, 12, 14-15 and 17-20 are rejected under 35 U.S.C. §112, second paragraph, as being indefinite for failing to particularly point out and distinctly claim the subject matter which applicant regards as the invention. In response, the claims have been amended in order to specifically address the Examiner's noted concern. Accordingly, this rejection is overcome.